DIRECT AND AFTER EFFECTS OF CHANGES IN MEDIUM DURING DIFFERENT PERIODS IN THE LIFE HISTORY OF *UROLEPTUS MOBILIS*.

I. EFFECTS OF BEEF EXTRACT.

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In 1905 Woodruff [1] as a result of his experiments with salt stimulation stated, "the conclusion seems to be justified that a given stimulus produces different effects at different periods in the life cycle." For the past two years while working on the problem of the nutrition of *Uroleptus mobilis*, I have had the opportunity of testing the sensitiveness of the protoplasm of different ages and find that so far as the division rate is concerned, the nature of the response to beef treatment and in all probability to treatment with potassium phosphate and other salts can be predicted if the age of the protoplasm is known.

The material used in these experiments was taken originally from the stock of Professor Calkins at Columbia, and the same series numbers were kept. From time to time conjugations occurred in the stock cultures at Barnard and new series were started in which case the letter *B* has been added to the series number to distinguish it from the Columbia series of the same number but possibly not of the same ancestry. In the present paper the results are given of experiments with beef extract only. In a later paper, the experiments with potassium phosphate and other salts will be reported.

A. The Direct Effects of Beef Feeding.

Professor Calkins [2] has cultivated *Uroleptus mobilis* for the past six years on a hay-flour medium, made "by boiling 100 mgs. chopped hay with 130 mgs. flour in 100 cc. Great Bear Spring water for ten minutes and diluting this when 24 hours old with equal parts of spring water." Woodruff and Baitsell [3] found that a .025 per cent. solution of Liebig's beef extract was a favorable medium for certain infusoria, and after experimenting with solutions of varying strengths, this percentage has been used in

all of the following experiments with beef extract. A .05 per cent. solution of beef extract made up with Great Bear Spring water and a 24-hour-old solution of flour water of the same strength as in the normal hay-flour medium of Calkins, were used in equal amounts. This resulting medium was a solution of the most favorable beef strength and exactly similar to the normal medium except that the beef solution had been substituted for the hay.

In conducting the experiments one individual was taken from each of the five lines of the control series and placed in the beef flour medium. Both series were kept under the same conditions of light and temperature. They were transfered to fresh medium at the same times and differed only in the hay or beef element of the medium. As in all similar experiments the rate of division was taken as the indication of the vitality of the protoplasm.

Discussion of Experiments.

(*a*) *Direct Response of Young Protoplasm to Beef Extract.*

Table I. shows the results of feeding very immature series with the beef-flour medium. Six young series of five lines each were tested for their reaction to the substitution of beef extract for the hay in normal medium and in every case there was a lowering of the vitality indicated by the slowing of the division rate. The amount of the depression varied from 1.8 to 9.6 divisions in ten days for each line of the series. Every young line responded in the same way indication that in this period which in the six cases extended from the 5th to the 40th generation, the protoplasm was in a condition in which the normal metabolic processes were easily and immediately upset and the vitality lowered.

Table I.

Effects of Beef Feeding during the Period of Immaturity.

Series No.	Age in Gen.	Av. No. Divisions per Line in 10-day Periods, Beef Series.	Av. No. Divisions per Line in 10-day Periods, Control Series.	Amt. of Depression in Division Rate per Line in 10 Days.
82....	17–24	11	21.6	9.6
97....	11–34	17.6	20.8	3.2
98 B..	17–40	20	24.8	4.8
99 B..	11–30	15.2	17.6	2.4
100 B..	11–25	13.2	15.0	1.8
101 B..	5–23	13.6	18.4	4.8

(*b*) *Direct Response of Older Protoplasm to Beef Extract.*

Table II. shows the results of feeding *Uroleptus* with the beef-flour medium when the protoplasm was older than in the experiments shown in Table I. Series 97, 98*B*, 99*B*, 100*B*, 101*B*, all of which had been depressed in the beef-flour medium when tested earlier in their life history, now are stimulated by the treatment. Series 97 during the 60–75th generations showed an increase in ten days of 6.8 divisions per line over the control series. The stimulating effect of the beef was seen again though to a less degree in the 167th generation and even in as late a period as the 225th generation, the beef series maintained a slightly higher rate of division than the control. Series 98*B* depressed in the 40th generation, was stimulated in the 75th and 122d generations and at both times, the division rate exceeded that of the control. This same increase it vitality was observed in varying degrees in Series 99*B*, 100*B*, and 101*B* each one having been previously depressed by the beef treatment. In addition to the above experiments, three other series were tested whose reaction to beef in their earlier history was not known. Series 72 was in its 50th generation when it was obtained from Columbia. A series was treated with beef for 40 consecutive days and throughout the entire period from the 50th to the 140th generation, the stimulating effects of the beef were apparent. Even in the 180th generation the division rate of the beef series was equal to that of the control. Series 81 during the 56th–84th generation, was treated with beef for 20 consecutive days. During the first ten days the division rate was slightly less than that of the control. In the second ten period, however the beef series showed a more rapid division rate than the control. Series 95 was treated for 30 consecutive days, from the 50th–102d generation and at no time was there any evidence of stimulation. When the series was again tested in the 160th, 190th and 210th generations, the beef series responded with a much higher division rate than the control. Thus all eight series in their later life history responded to beef treatment with an increase in the vitality as indicated by a quickened division rate. The age at which this increase appears varies as would be expected. Series 99, 100, 101, all divided more rapidly in the beef medium at an early age, one as young as

27 generations. Series 72, 97, 98 showed the stimulating effects of the beef about the 60th–75th generation. Series 81 and 95 failed to respond to the beef until a still later age and might be considered slow maturing series. Series 95 gave indications of a long period of immaturity, showing distinct depression in the beef as late as the 102d generation. Beef apparently is a depressant in the early generations and a stimulant in later periods of the life history.

TABLE II.

THE EFFECTS OF BEEF FEEDING DURING A LATER PERIOD IN THE LIFE HISTORY.

Series No.	Age in Gen.	Av. No. Divisions per Line in 10-day Periods, Beef Series.	Av. No. Divisions per Line in 10-day Periods, Control Series.	Amt. of Increase in Division Rate per Line in 10 Days, Beef Series.
72	58–79	19.8	18.4	1.4
"	79–100	20.6	19.2	1.4
"	100–125	17.0	14.0	3.0
"	125–140	16.8	9.2	5.6
"	180–200	13.6	13.6	0.0
81	56–68	16.0	16.4	−0.4
"	68–84	16.0	14.0	1.4
95	50–70	18.0	18.0	0.0
"	70–84	14.0	16.0	−2.0
"	84–102	12.0	18.0	−6.0
95	160–175	19.6	16.6	3.0
"	190–210	22.4	17.8	4.6
"	210–225	20.8	15.7	5.1
97	60–75	21.6	14.8	6.8
"	167–185	16.2	15.2	1.0
"	235–254	11.4	11.2	.2
98 B	75–97	19.0	17.8	1.2
"	122–136	16.0	14.0	2.0
99 B	40–60	17.4	14.4	3.0
"	75–90	13.4	12.6	.8
"	95–105	11.6	11.8	.2
100 B	27–58	17.4	16.4	1.0
"	90–105	13.0	11.6	1.4
101 B	39–64	14.2	11.0	3.2
"	80–102	14.0	13.8	.2

(*c*) *Direct Response of Old Protoplasm to Beef Extract.*

Table III. shows the results of feeding old series with the beef-flour medium. Eleven experiments were carried out on protoplasm varying in its age from the 210th to the 370th generation

and in every case the result was one of depression and a lowering of the vitality. Series 61 taken from Columbia stock in the 250th generation was depressed to such an extent in the beef-flour medium that its division rate was cut from 16.4 divisions in ten days to 7.6 divisions in ten days and during the next ten day period the series died while the control was dividing normally. Series 69 was treated for 40 days when the protoplasm was from 175–335 generations old, and at no time did the division rate of the beef series equal that of the control, the average rate per line for each ten day period being 9.8 divisions while that of the control series was 14.5. divisions. Series 95 failed to show indications of age until the 230th generation when the beef series divided in ten days on an average of 3.2 divisions less than the control. When this same series was again placed in the beef-flour medium in the 310th generation, the division rate fell to 6.0 divisions per line less than that of the control. Series 97 was tested for its reaction to beef six times in its life history and after the initial depression in the period of youth, the vitality was not lowered until the 260th generation. From this age on, there was a definite slowing of the division rate for the beef, flour series. Series 61, 69, 77, 88 were all old series when taken from the Columbia stock so there was no opportunity to test them when younger. In their old age however, they gave the expected response to the beef-flour treatment.

A comparison of the results of these three sets of experiments indicates that the protoplasm of *Uroleptus* responds in a definite manner to treatment with beef according to its age. The very young individual apparently does not adjust its self to the new medium, its metabolic processes are retarded and its vitality lowered. At a later stage in its life history it is able at least to adjust itself to the change in medium and to maintain a vitality as great as that of the control. Then appears an age when it is not only able to adjust its self to new conditions but is stimulated by them and shows an immediate definite increase in its division rate. Finally with age there is again a lack of adjustment indicated by the lowering of vitality and slow division rate. If the series is very old the substitution of the beef is sufficient to cause the death of the race.

TABLE III.

EFFECTS OF BEEF FEEDING DURING THE PERIOD OF OLD AGE.

Series No.	Age in Gen.	Av. No. Divisions per Line in 10-day Periods, Beef Series.	Av. No. Divisions per Line in 10-day Periods, Control Series.	Amt. of Depression in Division Rate, Beef Series.
61.....	360–370	8.8	16.4	7.6
69.....	275–335	9.8	14.5	4.6
72.....	200–225	11.2	12.0	.8
"	225–236	11.4	12.0	.6
77.....	210–230	13.8	16.2	2.4
88.....	255–270	7.6	8.8	1.2
95.....	230–243	10.6	13.8	3.2
"	280–290	6.2	8.0	1.8
"	305–310	0.8	6.8	6.0
97.....	260–270	5.4	7.2	1.8
"	270–283	2.6	6.6	4.0

No definite time can be set for the appearance of maturity or the onset of old age. If a definite favorable response to the beef treatment is an indication of maturity, it may occur as early as the 30th or as late as the 102d generation. For the average series it would appear about the 60th generation which agrees with the statement of Calkins that the period of youth or immaturity covers about the first 60 generations. Old age usually shows its self about the 225th generation but it may appear as early as the 200th or as late as the 260th generation. Old age and immaturity outwardly are alike in their response to beef treatment. They also seem to agree in their relative duration. If the period of youth is prolonged as in series 95 the period of old age is likewise longer than that of the average. Series 95 lived 350 generations and showed indications of old age during the last 120 generations, Series 97 matured in about the average time of 60 generations and showed evidences of age only in the last 40 generations, the race dying in the 300th generation.

B. The After Effects of Beef Feeding on Protoplasm of Different Ages.

In a number of experiments after a series had been kept in the beef-flour medium for ten days, one individual from each of the five lines of the series was put back into normal medium and the division rate of the new series compared with that of the series continued in the beef-flour medium.

Three young series after ten days in the beef medium were transfered to normal medium and in every case the vitality was increased to such an extent that it exceeded that of the beef series and equaled that of the normal control series. In other words the depression while in the beef medium was followed by a stimulation on the return to normal medium.

Four mature series were treated in the same manner some twice and others three times, and in one experiment only was there any indication of a stimulus when the series was transfered from the beef to normal medium. In five experiments the transfer was followed by a distinct lowering of the division rate and in three other experiments, there was no change in vitality as indicated by the division rate.

Finally series 95 and 97 were tested twice each in their old age and in all four experiments the transfer to normal medium was followed by a distinct increase in vitality.

Here again is seen the similarity in the behavior of immature and worn out protoplasm. In both cases there is an immediate depression of the life processes when treated with the beef, but this depression disapears on removal to normal conditions and in some cases there follows a period of higher vitality, the real effect of the beef being only delayed in its appearance. On the other hand the mature individuals respond immediately with a more rapid division rate which falls when the stimulus is removed and the medium is again normal. Immature protoplasm and protoplasm of old age are apparently not able to adjust themselves quickly to changes in the environment. They are at once depressed in the new condition but overcome the depression when back in normal medium and often show a quickened vitality. Mature protoplasm is like a healthy muscle that is always in tone, ready to respond to a stimulus and needs no time

for adjustment to the changed conditions, thus the effects appear at once and would not be expected to continue when the cause has been removed. It may be concluded that beef is always more or less of a stimulant to Uroleptus but the time when the stimulating effects are apparent varies with the age. Mature races will respond at once. Immature and old series are at first depressed and show the stimulating effects only after they are once more in their normal environment.

TABLE IV.

AFTER EFFECTS OF BEEF FEEDING.

Series No.	Age While in Beef.	Av. No. Divisions per Line in 10-day Periods, Transfered Series.	Av. No. Divisions per Line in 10-day Periods, Beef Series.	Effect of Change from Beef-Flour to Normal Medium.
82	17–24	18.0	5.4	Stimulation
99 B	11–30	16.8	16.2	"
101 B	24–34	15.2	13.8	"
98 B	75–97	15.6	16.0	Depression
"	122–136	12.6	16.0	"
"	158–170	11.2	12.2	"
99 B	40–60	15.6	15.6	No effect
"	80–105	11.0	11.6	Depression (slight)
"	105–120	10.8	10.2	Stimulation (slight)
100 B	37–58	14.2	14.2	No effect
"	90–105	10.6	13.0	Depression
101 B	39–64	14.0	14.0	No effect
95	280–290	8.2	7.8	Stimulation
"	305–310	5.9	0.8	"
97	235–255	8.0	7.4	"
"	270–84	8.2	2.6	"

SUMMARY.

The response of *Uroleptus mobilis* to treatment with a beef-flour medium varies with the age of the protoplasm. Both young and old series are immediately depressed in the beef medium but recover their vitality and may show a greater division rate than that of the control series when transferred to the normal hay-flour medium. Mature series on the other hand are stimulated when placed in the beef medium. The stimulation however is not

continued when the series is returned to the normal environment. Beef seems to be an immediate stimulant if the protoplasm is mature, and a delayed stimulant if the series is young or old, the effect being seen only after transference to normal hay-flour medium.

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